1991 NATIONAL WORKSHOP

BARLEY YELLOW DWARF DISEASE OF CEREALS CAUSED BY BARLEY YELLOW DWARF VIRUS

NOVEMBER 17-18, 1991

SCHAUMBURG, ILLINOIS

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE

EXECUTIVE SUMMARY

A national workshop on barley yellow dwarf disease and barley yellow dwarf virus (BYDV) was organized by the United States Department of Agriculture, Agricultural Research Service and was held in Schaumburg, Illinois, November 17-18, 1991. Similar workshops were held in 1977 and 1988. The overall goal of the workshop was to assess the current status of the national (state and federal) research program on the barley yellow dwarf disease problem in general and BYDV in particular.

The specific objectives of the 1991 workshop were to:

(1) review the current status of barley yellow dwarf disease and BYDV;

(2) review the current status of BYDV research since the 1988 workshop;

(3) identify current research gaps and needs in the national research program; and

(4) verify existing and/or establish new research priorities in the national research program.

The general purpose of these workshops is to ensure a coordinated national research program to reduce unnecessary duplication of effort among state and federal scientists in order to enhance progress on the problem. In addition, it is expected that these workshops will encourage interaction and cooperation among scientists working on the barley yellow dwarf disease problem.

Twenty-two participants took part in the workshop discussions. The participants included state (SAES) and federal (ARS) scientists, as well as representatives from Canada and Mexico (CIMMYT), currently working on (or with an interest in) barley yellow dwarf disease and the BYDV problem. In addition, representatives from the Barley and Oat National Improvement Committee participated in the workshop.

Current research is focused on:

-- BYDV strain identification, differentiation, and detection;

-- structure and organization of the BYDV genome;

-- regulation of viral gene expression;

-- BYDV-cereal host interaction(s);

-- BYDV coat protein-mediated cross protection;

-- application of molecular biological approaches to host resistance;

-- mechanism(s) of BYDV transmission by insect vectors;

-- insect vector behavior;

-- integrated pest management, including chemical and biological control of insect vectors of BYDV, cultural practices and host resistance;

-- germplasm enhancement, evaluation and selection for BYDV resistance or tolerance;

-- identification of specific genes for, and new or novel sources of, resistance or tolerance to BYDV; and

-- improved methods to select for BYDV resistance or tolerance.

Generally, most of the research needs identified in the 1988 BYDV workshop are currently being addressed by ARS and SAES scientists. Nevertheless, some current information gaps and research priority needs were identified. These include:

-- improved methods for detection and identification of BYDV strains or variants in mixtures of strains;

-- additional and/or improved BYDV diagnostic reagents;

-- better understanding of BYDV strain variation;

-- better understanding of host resistance and tolerance to BYDV infection based on virus-host interactions;

-- identification, characterization and mobilization of broad-based BYDV resistance genes;

-- better understanding of virus and vector ecology and epidemiology;

-- development of innovative biotechnological strategies and increased application of molecular biological approaches for stable resistance to BYDV infection;

-- better understanding of viral and host resistance/tolerance gene regulation;

-- transformation system(s) for oats and barley;

-- improved integrated strategy based on BYDV epidemiology, insect vector, ecology, host resistance, cultural practices, and insect vector control, including biological control, to manage barley yellow dwarf disease.

Edwin L. Civerolo
National Program Leader
Plant Health

Charles F. Murphy
National Program Leader
Grain Crops

December 1991

A. INTRODUCTION:

In 1987, ARS developed a comprehensive plan of research on the barley yellow dwarf disease problem. This plan was reviewed by scientists in the United States, including members of the three National Cereal Improvement Committees.

The overall goal of the research program is to develop and transfer to cereal grain producers practical technology to prevent or minimize economic losses due to barley yellow dwarf disease. Two major research objectives to achieve this goal are to:

(1) develop small cereal grain germplasm and new varieties with stable resistance or tolerant to barley yellow dwarf virus (BYDV) infection and

(2) develop small cereal grain crop production systems involving cultural practices, BYDV-resistant or -tolerant varieties, and suppression of insect vector populations.

The first national workshop on barley yellow dwarf disease was held in Urbana, Illinois, June 1-2, 1977. A major outcome of that meeting was a recommendation that a coordinated national state/federal research effort be developed to address the BYDV problem.

The second national workshop on barley yellow dwarf disease was organized by the United States Department of Agriculture, Agricultural Research Service, and was held in Chicago on March 22-23, 1988. The objective of this workshop was to identify priority research needs and gaps in the national program.

A third national workshop on barley yellow dwarf disease of cereals, also organized by ARS, was held November 17-18, 1991, at the Woodfield Hyatt Regency Hotel in Schaumburg, Illinois. The specific objectives of the 1991 workshop were to:

(1) review the current status of barley yellow dwarf disease and BYDV;

(2) review the current status of BYDV research since the 1988 workshop;

(3) identify current research gaps and needs in the national program; and

(4) verify existing and/or establish new research priorities in the national program.

The overall purpose of these workshops is to ensure a coordinated national research program to reduce unnecessary duplication of effort among state and federal scientists in order to enhance progress on the problem. In addition, it is intended that these workshops will encourage communication interaction and cooperation among persons working on the barley yellow dwarf disease problem.

Currently, there are approximately 25 state (SAES) and federal (ARS) scientists conducting research on various aspects of the barley yellow dwarf disease problem in the United States. The ARS research effort in FY 1992 consists of 4.1 SYs in seven locations. The total ARS funding in FY 1992 is approximately $636K.

Barley yellow dwarf disease is a broad, complex problem. Therefore, the topic was divided into three subareas for discussion purposes as in the 1988 workshop. These subareas were the (1) barley yellow dwarf virus (BYDV), (2) insect vectors, and (3) host crops.

The workshop was attended by 22 participants who took part in the discussions. A list of the participants is attached (Attachment 1). The participants included state (SAES) and federal (ARS) scientists, as well as representatives from Canada and Mexico (CIMMYT), currently working on (or with interest in) barley yellow dwarf disease and BYDV. In addition, representatives from the Barley and Oat National Improvement Committee participated in the workshop.

Each scientist provided a 1-page summary of his/her current research program describing the research objectives and approaches, status of the research, accomplishments since the 1988 workshop, limitations and constraints, and needs. These are attached at the end of this report (Attachment 2).

The workshop was held immediately before the Oat Biotech VI meeting sponsored by The Quaker Oats Company. ARS appreciates The Quaker Oats Company accommodating this workshop and assisting in making local arrangements.

B. DISEASE STATUS:

Significance: BYDV infects important cereals and grasses worldwide, including small cereal grains throughout the cereal growing regions of the United States. Epiphytotics occur sporadically under appropriate environmental conditions. Generally, economic losses in the United States due to BYDV directly or indirectly (through physiological responses to environmental stresses and to other pests and pathogens) range from about 1-5%; however, yield losses can exceed 60%.

Symptomatology: Symptoms caused by or associated with BYDV infection of barley, wheat, rye, triticale and rice generally include dwarfing and leaf yellowing. Infected oat leaves become purple or reddened. Symptoms on infected maize include chlorotic spots or streaks on young leaves and premature purpling of basal leaves.

Epidemiology: The interactions between virus strain, aphid biotype, host cultivar, and environmental factors in disease occurrence are extremely complex. Perennial grasses and weeds may form the natural reservoir serving as sources of BYDV. Aphids introduce BYDV every year in autumn-sown cereal crops. Primary infection foci are scattered and sparse. Secondary spread of the virus occurs subsequently in the fall and particularly in the spring as aphids move, migrate, and feed.

Management Strategies: Barley yellow dwarf disease management is based on several strategies, including host resistance or tolerance, cultural practices, monitoring migrating aphid vectors and planting cereals to emerge after the autumn aphid vector population peak has passed, and timely appliation of insecticides. Increased knowledge of BYDV epidemiology is necessary to ensure the most effective management of the disease.

C. CURRENT RESEARCH:

1. The Virus

Barley yellow dwarf virus (BYDV) is a complex of virus variants that belong to the luteovirus group of plant viruses. BYDV variants are distinguished on the basis of specific or nonspecific transmission by different aphid species, serological relationships, and cytopathology. Serologically, BYDV is related to several luteoviruses. BYDV currently refers to the persistently-transmitted, serologically-related viruses that cause cereal diseases with similar symptoms and effects.

Current research on the virus(es) is focused on:

(1) strain characterization, variability, differentiation, and identification based on serology using poly- and monoclonal antibodies and cDNA probes;

(2) detection using molecular (e.g. cDNA) and chemiluminescent probes and polymerase chain reaction (PCR) amplification;

(3) genome structure, organization, and characterization, specifically with respect to regulation of coat protein synthesis, aphid transmissibility, and host range;

(4) viral gene expression regulation and strategies involved in translation of the BYDV genome, including the effect(s) of RNA structure on genome translation, increased expression of coat protein, and satellite-like RNA ribozyme;

(5) mechanism of how BYDV causes disease and how the host responds, including the subcellular location of virus proteins in infected cells, the identification of genes involved in resistance to infection, and mapping (via restriction fragment length polymorphisms and PCR) differences between susceptible and tolerant host lines;

(6) epidemiology and ecology; and

(7) development of a monocot protoplast assay system to assay the virus and to study viral gene expression and viral replication.

2. The Insect Vector

BYDV is transmitted by aphids in a persistent manner. Many species of aphids that are vectors of plant viruses infest Gramineae hosts; however, the relative importance of each species as a vector of BYDV depends upon its natural behavior and life cycle. Migrant aphids introduce BYDV into both spring and autumn-sown cereal crops each year.

Current research on the insect vectors is focused on:

(1) BYDV-vector relationships, including mechanism(s) of transmission;

(2) insect control/management with insecticides;

(3) relationship between aphid vectors of BYDV and Hessian fly;

(4) integrated pest management, including barley yellow dwarf disease; and

(5) biological control.

3. The Host

The range of BYDV hosts in the Gramineae is wide. In addition, perennial grasses in general are the main natural reservoir of BYDV. Barley yellow dwarf disease primarily affects wheat, oats, and barley. However, BYDV also infects triticale, rye, rice, and maize. The role of maize in the survival of aphid vectors and luteoviruses between spring and fall cereals is not fully understood. Host plant resistance or tolerance to BYDV is still the primary basis for effective integrated management of barley yellow dwarf disease. Major (Yd2) and minor (yd1) genes conditioning BYDV resistance occur in barley. Additional genes affecting host resistance to BYDV may also exist. Major genes for BYDV resistance have not been identified in wheat, triticale, or oats.

Current research on the host(s) is focused on:

(1) BYDV incidence surveys;

(2) inter- and intrafield spread of BYDV, including methodology for measuring BYDV spread in the field;

(3) identification of sources of BYDV and aphid vectors;

(4) germplasm screening/evaluation and selection for BYDV resistance;

(5) development of crop loss models;

(6) host resistance or tolerance to BYDV, including incorporation of BYDV resistance/tolerance into adapted germplasm and improved cultivars, development of interspecific hybrids, identification of the number of genes involved, and identification of molecular markers associated with BYDV resistance/tolerance;

(7) analyses of existing, and identification of, new sources of BYDV resistance/tolerance and introgression of these into adapted genotypes;

(8) mechanisms of host resistance or tolerance to BYDV;

(9) cross-protection mediated resistance to BYDV;

(10) development of improved methodology to select for BYDV resistance/tolerance in order to eliminate BYDV-susceptible genotypes as early as possible; and

(11) development of pairs of near isogenic lines with differential BYDV tolerance.

D. BYDV RESEARCH NEEDS

Generally, most of the research needs identified in the previous BYDV workshop in 1988 are being addressed by ARS and SAES scientists. Nevertheless, some current information gaps and research needs were identified by the workshop participants. These include:

(1) improved methods for detecting and identifying BYDV strains or variants in mixtures of strains;

(2) better understanding of BYDV strain variation with respect to genome sequence, differential host specificity, vector specificity, nature or basis, eco- or biotype, genome structure organization, and viral gene expression;

(3) a transformation system and plant regeneration system for oats and barley (as in rice and wheat);

(4) "pure" isolates based on infectious clones of BYDV variants, e.g. serotypes;

(5) better understanding of virus and vector ecology and epidemiology within and among fields, effect(s) of host on source or virulence of aphid vectors, and inoculum sources;

(6) improved methods for preservation of BYDV variants;

(7) additional and/or improved BYDV diagnostic reagents (i.e. antibodies, molecular probes);

(8) broad spectrum coat protein-mediated cross protection, at least among strains within a serotype;

(9) development and application of other molecular biological approaches for stable resistance to BYDV infection, including, but not limited to, ribozymes and antisense RNA;

(10) identification and characterization of broad based BYDV resistance genes, including a better understanding of their regulation, and incorporation of these into commercial varieties;

(11) better understanding of BYDV-cereal host interactions with respect to how the virus affects its host(s) and is related to resistance/tolerance or susceptibility;

(12) additional information about insect vector biology and behavior, significance of other aphid species in BYDV transmission, seasonal distribution and behavior of aphid vectors, vector survival, epidemiological significance of host resistance to aphid vectors, and mechanism(s) of BYDV transmissibility by insect vectors;

(13) better understanding of the epidemiological significance of BYDV-tolerant and -resistant hosts;

(14) innovative methods to reduce costs and to be more efficient for germplasm evaluation and selection; and

(15) increased integrated management, including biolgical control, of aphid vectors of BYDV.

E. RESEARCH NEEDS AND PRIORITIES:

The participants did not identify research priorities based on the needs listed above. However, based on the overall discussion, the following generalizations can be made.

(1) The most effective strategy for management of barley yellow dwarf disease is based on host tolerance of all BYDV variants. Stable tolerance is better than unstable resistance. Quantitative trait loci and gene pyramiding approaches to developing BYDV tolerance are viable strategies.

(2) There is a need to identify, characterize, and mobilize host and viral genes that function in BYDV tolerance and resistance.

(3) Biotechnology-based strategies or molecular biology approaches to developing BYDV-tolerant or -resistant hosts need to continue and expand. These include, but are not limited to, synthetic resistance genes that interfere with viral replication, coat protein-mediated resistance, mutant or modified relicase(s) and ribozymes.

(4) New molecular biology techniques should be used to more precisely characterize variant BYDV strains for improved virus strain detection and identification.

(5) Increased research on BYDV-cereal host interaction(s) is needed for a better understanding of BYDV resistance and susceptibility.

(6) The current management of barley yellow dwarf disease is an integrated approach based on host resistance, cultural practices, and insect vector control. Continued and expanded research on the ecology of the virus and insect vectors, and on the epidemiology of the disease in general, is needed.

(7) The current research effort on biological control of aphid vectors of BYDV is limited. This effort should be increased.

APPENDIX #1

1991 BYDV WORKSHOP PARTICIPANTS

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APPENDIX #2

RESEARCH SUMMARIES FOR 1991 BYDV WORKSHOP

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International Maize and Wheat Improvement Center (CIMMYT).
Apdo Postal 6-641, 06600
Mexico DF

Projects

1. Screening bread wheat , durum wheat, triticale, barley and wide cross germplasms for resistance to barley yellow dwarf viruses (BYDVs) under natural infections in the field.

2. Studying the genetics of resistance to BYDVs and evaluating progenies from selected crosses for their resistance to BYDV.

3. The effect of BYDVs on the yield of cereals in Mexico.

4. Trapping live cereal aphids and determining the percentage that are able to transmit BYDVs.

5. Variation in serotypes of BYDVs in cereals in Mexico.

6. Survey of BYDV serotypes in cereals in Latin America, Asia and Africa.

7. Assessment of titres of BYDVs in "field resistant" barleys, wheat and wide crosses.

8. Cross protection studies with BYDVs.

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(Also assoc. professor at Laval University)

Research objectives and approaches:

Germplasm evaluation and development. Since 1971, I have evaluated germplasm for BYDV tolerance in oats, barley and wheat, for breeding purposes. After the beginning of the IDRC project with Dr. St-Pierre (Laval U.) in 1983, the research expanded more into bread wheat, durum, triticale and gradually decresased in oats. The best sources of tolerance were evaluated in crosses, to measure heritability.

Service to breeders. We selected from bulk populations the lines that the best tolerance, and assessed breeder's lines in pre-registration trials or registration trials.

New germplasm from interspecific hybrids. Oat interspecific hybrids were found promising as sources of tolerance in 1977-83. A long, frustrating search for resistance genes in conventional durum wheat and bread wheat led to the conclusion that interspecific hybrids were needed. Gradually we tried more difficult hybrids with novel media and methods. This also led to haploid production; for example wheat x maize generates haploids.

Status of research. We now move into a study of drought x BYDV interactions, and on possible uses of BYDV as a tool to improve drought tolerance. Evaluation and selection of germplasm remain as minor goals. We also developed an interspecific germplasm bank of crosses of wheat to 27 species of Triticeae; this germplasm will be evaluated and selected first with BYDV, and later on under drought. Development of new biotechnological tools has become a key aspect. We want to try transposon tagging to study the BYDV resistance genes of the Triticeae.

Other. We have developed associations with scientists from France, and currently one of our grad students is sponsored by Germany. This sometimes creates limitations to the diffusion of technology and germplasm, but we have no choice but to learn the new rules of the game. We need to expand in the use of ELISA and molecular genetics, and research partnerships might be welcome in these areas.

1991 Barley Yellow Dwarf Virus Workshop

Cleora J. D'Arcy
Department of Plant Pathology
University of Illinois, Urbana, IL

Research Objectives and Approaches:

Work on barley yellow dwarf viruses is part of a larger project involving members of the luteovirus group. For that group, objectives are to produce serological reagents useful for detection and diagnosis, to determine the nature and location of viral epitopes, and to examine the usefulness of monoclonal antibody-based assays in large-scale field studies. Specific objectives of research on BYDVs include study of virus-host interactions and mechanism(s) of resistance and study of gene expression of the virus. Most of the approaches involve serology, utilizing both polyclonal and monoclonal antibodies. Membrane fractionation, nucleic acid detection techniques and electron microscopy are other approaches taken.

Status of Research:

In 1989 and 1990 surveys of spring oat and winter wheat fields across Illinois, PAV- and RPV-like serotypes of BYDVs were detected with DAS-ELISA and TAS-ELISA. Agreement between the two assay systems was >99% for 900 and 1,050 samples in the two years. The overall incidence of PAV serotypes was higher than that of RPV serotypes (8.3% vs. 1.7% in 1989; 16.6% vs. 2.5% in 1990). Incidences of BYDVs were higher in oats (0-40% in 1989; 4-60% in 1990) than in wheats (0-2% in 1989; 0-60% in 1990). The results demonstrate the usefulness of monoclonal antibodies (MAbs) to highly conserved viral epitopes in field surveys for BYDVs. Survey samples from 1991 are being analyzed.

A panel of 14 murine MAbs to BYDV-MAV-NY have been produced and characterized. The MAbs are of two isotypes: IgG1 and IgG2a. Conformation sensitive immunoassays indicate that each of the MAbs recognizes a conformational epitope. Three of the MAV MAbs recognize PAV, while none recognize RPV. Several of the MAbs are effective trapping antibodies, allowing the development of totally monoclonal ELISA systems. The sensitivity and reliability of these systems are being compared to those of TAS-ELISA systems currently in use.

Yields of RPV-IL and PAV-IL purified from three oat cultivars have been compared with the titers of virus in these hosts as measured by ELISA. ELISA results indicated that each of the virus strains was present in shoot and root tissues of all oat cultivars, and that shoot tissue contained more virus than root tissue. Highest yields of RPV-IL were from roots of Coast Black oats. One-third to 1/2 as much virus was purified from shoots of any of the three cultivars, and no virus could be purified from roots of either Clintland 64 or California Red oats. Similar results were obtained for PAV-IL. These results indicate that caution is advisable in the use of ELISA results to predict amounts of BYDVs that can be purified from host tissues.

MAbs to PAV-IL detected virus particles in PAV-infected oat root endoplasmic reticulum (ER) fractions, but not in plasma membranes (PM) fractions. ELISA analysis of floatation gradients demonstrated that free virus particles were in the pellet, while virus associated with ER was collected at the 15%/27% sucrose interface. This phenomenon was observed with healthy oat root ER, but not with oat PM or red beet membranes. Anti-PAV polyclonal antibodies prevented membrane association. The results suggest existence of a host membrane component acting as a putative virus docking site.

1991 Barley Yellow Dwarf Workshop

Leslie L. Domier
USDA-ARS-Crop Protection Research Unit
Department of Plant Pathology, University of Illinois
Urbana, Illinois

Research Objectives and Approaches:

The barley yellow dwarf virus research in my laboratory is part at of group effort involving three virologists with different interests and areas of expertise. My primary objectives are to understand how BYDV induces disease in infected plants and how plant responses to virus infection manifest themselves as disease. Specific objectives include: (1) The identification of BYDV proteins in infected cells, their subcellular location and their interactions with cell components. (2) The identification and characterization of genes and genetic markers tightly linked to genes responsible for differential host response to BYDV infection. Approaches include: protein purification, antibody production, subcellular fractionation, cDNA cloning and gene mapping.

Status of Research:

To study the role of BYDV gene products in disease induction, antisera are being prepared to the products of each open reading frame in the BYDV-PAV-IL genome. cDNAs representing each open reading frame have been expressed in *Escherichia coli* as fusion proteins. The fusion proteins have been purified and injected into rabbits for the production of antisera. Work characterizing the individual antisera is in progress.

Near isogenic oat lines differing in response to BYDV infection have been produced by C. M. Brown and F. L. Kolb. These lines are being used to identify host genes responsible for differential response to infection. Genetic differences among the oat lines are being characterized at three different levels. Protein accumulation patterns are being examined by 2 dimensional protein electrophoresis. Differences in mRNA production among the oat lines are being examined through the production of subtracted cDNA libraries. In both cases, infected and non-infected oat plants are being examined since the genes of interest may not be expressed constitutively. Differences at the DNA level are being identified using RFLP and polymerase chain reaction techniques.

Anecdotal evidence suggests that more severe strains of BYDV are becoming more common. The annual surveys now being conducted in Illinois for BYDV incidence provide information on the prevalence of the different BYDV serotypes present within the state. Some of the BYDVs identified by this survey have been maintained in the green house. The PAV-like field isolates are being analyzed for differences in severity on test cultivars. In addition, the nucleotide sequences of these isolates are being compared to the laboratory strain of PAV-IL. These analyses will be repeated with 1991 field samples. These studies will allow us determine the genetic variability of PAV-like isolates from different geographical locations within Illinois and from different years as compared laboratory strain of PAV-IL.

BYDV PROGRAM SUMMARY

R. A. Forsberg[1/]
Dept. of Agronomy
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PROGRAM OBJECTIVES AND APPROACHES

Our effort is limited to multiple screening tests each year of advanced breeding selections of spring oats and soft red winter wheat:

OATS

1. Breeding nursery -- 10,000 lines.
   - rely on natural infection
2. Special late-planted test at Arlington[2/] -- 250 lines.
3. Illinois test -- 250 lines
   - viraliferous aphids applied

WHEAT

1. Breeding nursery -- 150 advanced breeding selections.

STATUS

Screening effort is constant from year to year. BYDV is a serious disease in Wisconsin, and continuous screening of germplasm is essential.

ACCOMPLISHMENTS

Have identified tolerant genotypes worthy of release as cultivars and have identified tolerant (resistant) breeding stocks worthy of use as parents.

LIMITATIONS

Lack of financial resources restricts the number of entries evaluated in the special Arlington test and in the Illinois test each year.

1/ Not present at Workshop.

2/ The special Arlington BYDV test is conducted at the invitation of Dr. A. H. Ellingboe, Plant Pathology, who has developed a nursery area heavily infected with soil pathogens and heavily infested with aphids. All infection is natural.

1991 Barley Yellow Dwarf Virus Workshop

Summary

Name: Roy French, USDA, ARS
Wheat, Sorghum, and Forage Research Unit
Lincoln, Nebraska

Research Objectives & Approach: The objective of my research is to determine the molecular mechanisms for interaction between viruses, vectors and host plants, particularly wheat, and implications of such interactions for controlling yield losses and enhancing profitability of wheat production. One step toward this objective has been to develop molecular-based plant virus detection methods for use in cereal virus epidemiology. Very little is known about the dynamics of cereal virus disease epidemics, especially for barley yellow dwarf virus (BYDV). This is due, in large degree, to the lack of efficient virus assay methods that can detect all of the different BYDV serotypes. The near-term goal was to devise techniques for diagnosing as many BYDV strains as possible.

A recent innovation, the polymerase chain reaction (PCR), provides an extremely sensitive means for detecting nucleic acid sequences. Regions of conserved sequences among potato leafroll virus (PLRV), beet western yellows virus, and the PAV isolate of BYDV were used to design a number of oligonucleotide primers for use with PCR.

Status of Research: One set of primers (spanning most of the coat protein gene) was found to work particularly well in diagnostic PCR. These primers can specifically detect PLRV, BWYV, subterranean clover redleaf virus (SCRV), BYDV isolates NY-PAV, MAV, RMV, RPV, and SGV, a Montana RMV isolate, as well as many isolates collected in the Northern Great Plains. Each luteovirus can be distinguished by characteristic restriction endonuclease cleavage patterns or by cross-hybridization with PCR amplified DNA from each virus. Field isolates were determined to be similar to PAV on the basis of such tests. This is the first technique that can simultaneously detect and identify all known BYDV strains as well as BWYV and PLRV.

A simple extraction procedure was developed using proteinase K digestion of plant tissue and it was found that such extracts could be diluted more than 1000-fold and still obtain a strong positive reaction, and weak positive reactions were detected at over 30,000-fold dilutions. As might be expected by the sensitivity of this assay, BYDV can also be detected in individual aphids by this PCR method. Costs can be reduced simply by using smaller reaction volumes.

In addition, two sets of degenerate PCR primers were developed from conserved amino acid sequences of putative polymerase genes of viruses in the carmovirus and sobemovirus groups. With these primers and PCR we have determined that BYDV MAV, SGV, and SCRV are, like PAV, similar to to carmoviruses in this region, and both RMV and RPV are similar to sobemoviruses. Degenerate primers derived from conserved amino acid sequences downstream of the coat protein gene have also been developed. By using different combinations of primers in PCR, over 2000 bases of luteovirus cDNA can be generated.

1991 Barley Yellow Dwarf Virus Workshop

Management Unit Representative: Stewart M. Gray
Management Unit: Plant Protection Research Unit
Location: Ithaca, New York
Objectives / Approach: The barley yellow dwarf virus (BYDV) program at Ithaca has two interrelated objectives: (1) to define the mechanisms of vector specific transmission of the viruses that cause barley yellow dwarf disease, (2) to investigate the mechanisms and epidemiological significance of plant resistance to BYDV and aphid vectors.

Vector specific transmission of BYDV involves the recognition of virus structural proteins by membrane receptors in the aphid salivary gland. Nontransmissible isolates of BYDV are acquired by any aphid, but are excluded from the salivary gland and therefore not inoculated into the plant. The biologically active regions of virus structural proteins are being identified by monoclonal antibodies that neutralize transport of the virus through the salivary gland membrane. Antigenic domains are mapped using a series of in vivo generated peptides representing overlapping regions of the viral structural proteins. Virus receptors present on aphid salivary gland membranes are being localized in immuno-electron microscopy studies utilizing anti-receptor antibodies and anti-receptor peptides.

Cereal genotypes are being evaluated for resistance to BYDV and aphid vectors. Currently spring oats are being utilized as the model plant system. Selection criteria include symptom expression, virus titer, yield components, and aphid transmission to and from the plant. The general mechanisms of virus resistance (e.g. reduced titer, localized distribution) are identified and evaluated in the laboratory and greenhouse as to their effects on virus transmission by aphids. The epidemiological significance of the various types of resistance mechanisms are then evaluated in field trials.

Status of Research: Ten monoclonal antibodies have been evaluated for their ability to neutralize virus transport in the aphid. Four antibodies inhibit transmission of one or more BYDV isolates. An additional 35 monoclonal antibodies are in various stages of characterization as to their specificity in binding to the NY isolates of BYDV and their ability to neutralize virus transport. Anti-idiotypic antibodies have been produced to 3 neutralizing monoclonal antibodies and are being tested for their ability to bind to membrane receptors. In addition, the capsid protein gene from two BYDV isolates has been cloned. Subclones have been used to produce, in a bacterial expression system, a series of overlapping peptides representing the entire capsid protein for both BYDV isolates. Using immunoblotting techniques the binding domains of 3 monoclonal antibodies have been identified.

A type of resistance manifested as a suppression of BYDV titer, measured by ELISA, was identified in a breeding line of oats (IL86-5262). Mean level of titer suppression, relative to a susceptible oat genotype ('Astro'), for 6 wks post-inoculation was 80%, 65%, 61%, 58%, and 3% for the RMV, PAV, MAV, SGV, and RPV isolates of BYDV, respectively. Transmission efficiency of PAV, MAV and SGV by single aphids was lower from the resistant tissue relative to susceptible tissue, but remained above 70%. Transmission efficiency of RMV dropped from >80% to <40%. In field trials, the incidence of RMV reached 90% in susceptible 'Astro' plots, but remained <1% in IL86-5262 plots despite heavy inoculum and vector pressure. Final disease incidence of the MAV isolate was 74% and 97% in resistant and susceptible plots, respectively. The resistance in IL86-5262 is BYDV isolate specific and emphasizes the need to use multiple isolates when screening for resistance.

1991 Barley Yellow Dwarf Workshop

Adrianna D. Hewings
USDA ARS Crop Protection Research Unit
Department of Plant Pathology, University of Illinois
Urbana, IL

Research Objectives and Approaches:

Barley yellow dwarf virus research in the cereal virus group at Urbana has several interrelated objectives: To investigate mechanisms of resistance to BYDV, to investigate cereal virus epidemiology; to determine the incidence, distribution and variability of BYDV virus strains across space and over time in oat, wheat and maize fields and to evaluate the response of cereal germplasm to economically important viruses and cooperate in cereal germplasm enhancement programs.

Status of Research:

Data from 1989, 1990 and preliminary data from 1991 indicate that BYDV spread occurred more rapidly in fields where infection foci were established prior to aphid infestation but the primary source of BYDV inoculum appears to be without rather than within the fields. Suction traps were used to capture live aphids crossing oat fields at canopy level. The first large aphid flights occurred in late April to early May in 1991, mid-May in 1990 and late May to early June in 1989. *Rhopalosiphum padi*, vector of both the BYDV PAV- and -RPV-like viruses was captured with greater frequency in 1990 than 1989. Incidence of BYDV in the Urbana field plots and in the state-wide survey was greater in 1990 than 1989. 1991 data are being analyzed.

The effects of time after inoculation of selected oat cultivars on their subsequent suitability as sources for BYDV transmission by aphid vectors is in progress. Don (moderately sensitive), Noble (moderately tolerant) and Ogle (tolerant) were selected for study because of their differential response to an Illinois isolate of a PAV-like BYDV. Transmission from Don oats was lowest 2 days after inoculation (2%). An increase in infected test plants was observed on day 5 that peaked at day 23 (61%). After day 23, transmission rates dropped; moderately efficient transmission continued until day 40 (22%). Transmission patterns for the Noble oat experiments were similar to the Don experiments except the rate of transmission was generally lower.

Ten maize inbreds that are widely used for hybrid production in the Midwest, A619, A632, A634, B68, B73, B84, CM105, Mo17, Pa91, and Va22 were tested for sensitivity to BYDVs in the greenhouse and in the field. No inbreds were infected with MAV-NY or RPV-NY. PAV-IL was found in 21-70% of A619, A634, B73, and B84 shoot and root samples. About 20% of the Pa91 root samples were positive. Preliminary studies to test PAV-IL transmission from barley to maize, maize to maize, and maize to barley suggest that *R. padi* transmits the virus with difficulty, if at all, from maize to maize but relatively easily from barley to maize and maize to barley. Recently an RMV strain has been isolated from commercial fields in both Minnesota and Illinois. Studies are underway to determine the importance of this strain in small grains and the role corn may play in BYDV epidemiology the Midwest.

1991 Barley Yellow Dwarf Workshop

Frederic L. Kolb
University of Illinois

Research Objectives and Approaches:

One of the primary objectives of the small grains breeding program is the integration of BYDV tolerance into adapted germplasm and improved varieties. This includes the development of genotypes combining BYDV tolerance with resistance to other diseases. A second objective is the identification of new sources of BYDV tolerance and introgression of the genes for BYDV tolerance into adapted genotypes including efforts to identify BYDV tolerant accessions of *Avena sterilis* and *A. fatua* that have not been used as sources of BYDV tolerance. A third objective is to develop methods to improve selection for BYDV tolerance in oats including evaluation of selection in early generations in the greenhouse. Another objective is the development of pairs of near-isogenic lines of oats differing in BYDV tolerance using several sources of tolerance to BYDV. These lines will be quite useful as tools in many research projects on BYDV, including the identification of molecular markers associated with genes for tolerance to BYDV.

Research Status:

Two experiments were conducted on methods of selection for BYDV tolerance in segregating populations grown in the greenhouse to determine if BYDV susceptible genotypes can be eliminated from populations by selection in the greenhouse. Effective selection would increase the efficiency of the breeding program since the frequency of BYDV tolerant genotypes in later generations would be higher. In one experiment, selection was somewhat effective in susceptible x tolerant crosses, but was not effective in tolerant x tolerant crosses. Evaluation of a second selection method is in progress.

In another experiment, selection for head weight in wheat with and without barley yellow dwarf virus infection is being evaluated to assess whether unthreshed head weight can be used to indirectly select genotypes with improved BYDV tolerance or higher yield potential. Evaluation of the populations resulting from selection will begin in 1992.

An experiment to determine if a modified recurrent selection method using a chemical hybridizing agent was effective in changing the frequency of BYDV tolerant genotypes in a wheat population has been completed. The technique did not change the frequency of BYDV tolerant genotypes in the population studied.

The development of near-isogenic lines differing in BYDV tolerance is in progress. Four backcrosses to the recurrent parent have been completed.

Identification and introgression of new sources of BYDV tolerance from *A. sterilis* and *A. fatua* is just beginning. A study of the inheritance of BYDV tolerance in *A. sativa* is in progress.

Research Summary: 1991 Barley yellow dwarf virus workshop - USDA/ARS

Name: Richard M. Lister

Institution and location: Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907-1155.

Research objectives and approaches: Growth in interest in barley yellow dwarf viruses (BYDV) in the small grain breeding program at Purdue dates from the late 1950's when pandemic outbreaks occurred in oats. By the mid 1970's, close attention was also being paid to breeding for BYDV resistance (or tolerance) in wheat. Virological work as such received its initial impetus from the needs of the breeding program and developing methods for BYDV control. It accelerated after the discovery in joint work with Bill Rochow (Cornell) that ELISA is sensitive enough to detect, differentiate and assay BYDV strains in crude plant extracts. Early efforts concentrated on virus purification and the development of serological reagents for comparative diagnosis and assay. More recently, cDNAs were also developed for these purposes, leading to comparative studies of the genomic sequence and organization of BYDVs and other luteoviruses. We have also studied BYDV epidemiology and ecology, work closely with the plant breeders in investigating resistances, especially among progenies from wide crosses (wheat x Thinopyrum spp.), and currently we are stressing the bioengineering of virus coat protein-mediated resistances. Future work will also emphasize ecological studies and studies of genomic function in collaboration with Dr. Mark Young, who recently joined the Department of Biological Sciences.

Results: Include those listed in the bibliography below.

Other comments: (1) Our main constraint is the difficulty of maintaining continuity as our work is absolutely predicated on funding that has to be sought piecemeal from various sources and at frequent intervals (1-2 years). One result is that the basic infrastructure required for virus-vector work available to us (i.e. dedicated greenhouse, growth chamber and insect transfer space) is poor, especially now that Dr. John Foster, previously USDA/ARS, has transferred to Nebraska. Against this, however, laboratory facilities are excellent, and we have been fortunate in assembling enthusiastic personnel and even growing in biomolecular expertise available to the program. (2) I take this opportunity of recognizing valuable support and encouragement for the Purdue program from several colleagues, especially Bill Rochow and Cornell.

Relevant papers published or in press since 1988:

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Pereira, A. M. N., and R. M. Lister. 1989. Variations in virus content among individual leaves of cereal plants infected with barley yellow dwarf virus. Phytopathology, 79:1348-1353.

Pereira, A. M. N., R. M. Lister, D. J. Barbara, and G. E. Shaner. 1989. Relative transmissibility of barley yellow dwarf virus from sources with differing virus contents. Phytopathology, 79:1353-1358.

Fattouh, R. A., P. P. Ueng, E. E. Kawata, D. J. Barbara, B. A. Larkins, and R. M. Lister. 1990. Luteovirus relationships assessed by cDNA clones from barley yellow dwarf viruses. Phytopathology 80:913-920.

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7 November 1991

Barley yellow dwarf virus research in the lab of

W. Allen Miller

Plant Pathology Dept., Iowa State University

Research Objectives and Approaches. We are studying two aspects of BYDV molecular biology: regulation of translation of the viral genome, and structure-function relationships of the ribozyme in a satellite RNA of BYDV. We predicted that BYDV uses the following strategies in translation of its genome: *(i)* frameshifting, *(ii)* initiation at more than one start codon on the same subgenomic mRNA, and *(iii)* readthrough of a stop codon . We are using the following approaches to study these events: *(i) in vitro* translation of *in vitro* transcripts, *(ii)* transient expression assays in protoplasts using expression vectors containing the translational control sequences in front of a reporter gene, so that the translational event of interest must occur for the reporter gene to be expressed, and *(iii)* inoculation of protoplasts with full-length infectious transcripts from cDNA clones of the viral genome that contain mutations that affect translation as determined in the above two approaches.

Status of Research. Using these approaches we found that a region in the viral polymerase gene induced a low level of frameshifting as expected. To our knowledge, this is the first demonstration of translational frameshifting in plant cells. We identified some sequences that are required for frameshifting and others that are not. We have precisely mapped the large subgenomic RNA of BYDV-PAV, synthesized it in vitro, and shown that the coat protein gene, an overlapping 17K ORF, and a 50K ORF translated by read-through of the coat protein stop codon are all translated from this subgenomic mRNA *in vitro*. We are determining the structures and sequences that regulate frameshifting, read-through, and the internal initiation required for 17K expression. We have optimized methods for inoculating oat protoplasts with BYDV RNA, which greatly simplifies our research. We have constructed a full-length clone from which transcripts can be derived but we don't know if they are infectious yet.

A self-cleaving satellite RNA was discovered in an RPV isolate of BYDV (Miller *et al., Virology,* 183, 711-720, 1991). Its effects on symptoms are unknown. We are studying a self-cleavage site in the RNA to better understand satellite replication, and to design gene-targeted ribozymes against BYDV. We have shown that the self-cleavage site in the satellite RNA prefers to fold into a novel pseudoknot-like structure rather than in the functional self-cleaving hammerhead structure (Miller and Silver, *Nucleic Acids Res.* 19, 5313-5320). After mutagenizing the sequence so that the hammerhead was the most stable conformation, the self-cleavage rate increased hundreds-fold. Future work will focus on the biological structure-function relationships of the satellite RNA. To do this, we have constructed a permuted dimeric clone from which full-length, correctly terminated satellite RNA can be transcribed. We will attempt to co-infect protoplasts with wildtype and mutant forms of the satellite and helper virus RNA, in order to map sequences required for replication. Finally, we hope to construct ribozymes which will specifically cleave BYDV genomic RNA. Genes encoding these will be introduced into BYDV hosts in collaboration with other labs skilled in cereal transformation.

RESISTANCE AND TOLERANCE TO BARLEY YELLOW DWARF VIRUS IN WHEAT AND OATS

Herbert Ohm and Hari Sharma
Purdue University

OBJECTIVES AND APPROACHES:

1. Develop oat and wheat germplasm lines and cultivars that have high levels of tolerance to BYDV (collaboration with Greg Shaner).

   Combine tolerance to BYDV from various source lines of Avena sativa and wild oat species (especially A. sterilis and A. strigosa) by pedigree and backcross breeding methods. Evaluate all yield nursery entries for BYD symptom severity in replicated hill plots and in yield nursery plots. Hill plots of 12 to 15 plants are infested with viruliferous Rhopalosiphum padi aphids in controlled environment chambers and transplanted to the field (collaboration with Roger Ratcliffe). A noninfested control hill plot is placed between two infested hill plots for all oat and wheat entries. Evaluations for BYD severity in yield nurseries are based on virus infection by natural populations of aphids.

2. Transfer resistance to BYDV from wheatgrasses to common wheat through wide crosses.

   Plant reactions to BYDV infection are based on ELISA (collaboration with Richard Lister) coupled with cytological analyses.

STATUS OF RESEARCH:

High levels of tolerance to BYDV have been transferred into several advanced oat lines with resistance to crown rust and one line, 7971A1-15-3-6 is being released as a cultivar. High levels of tolerance to BYDV have been transferred into several advanced wheat lines with other desirable traits.

Alien addition lines of common wheat that have resistance from Agropyron species (Thinopyrum intermedium, Th. ponticum, and Th. trichophorum) are being developed.

Name: Janine E. Powell

Institution: USDA, ARS, NPA, Northern Grain Insects Research Laboratory,
Rural Route #3, Brookings, South Dakota 57006

Research Objective: Plant Productivity - Develop technology for increasing plant productivity and quality.

Research Approach: Plant Protection - Control pathogens, nematodes, insects, mites and weeds to sustain or improve production efficiency in ways that will maintain or enhance natural resources and the environment.

Status:

A. Determine the influence of habitat and land use patterns on the species composition and abundance of cereal aphid predators in the principal field crops (small grains, alfalfa, corn) of the Northern Plains.

Cereal aphid predator populations were intensively sampled in small grain, alfalfa, and corn fields in eastern South Dakota during the 1988-1990 growing seasons. Crop characteristics (plant height, growth stage, stand density, plant species diversity), prey population density, and physical environmental measurements (air temp., relative humidity, wind velocity, light intensity) were taken in the field at the time of predator sampling. Land use patterns in the section surrounding the sampled field were recorded using ASCS maps. Data are being analyzed to determine the influence of these environmental factors on the abundance and diversity of cereal aphid predators at the sites. Results will be a component of a comprehensive IPM for cereal aphids.

Elliott, N. C. and R. W. Kieckhefer. 1990. A thirteen-year survey of the aphidophagous insects of alfalfa. The Prairie Naturalist 22:87-96.

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Elliott, N. C., R. W. Kieckhefer, and W. C. Kauffman. 1991. Estimating adult coccinellid populations in wheat fields by removal, sweepnet, and visual count sampling. Can. Ent. 123:13-22.

Kieckhefer, R. W. and N. C. Elliott. 1990. A 13-year survey of the aphidophagous coccinellidae in maize fields in eastern South Dakota. Can. Ent. 122: 579-581.

B. Determine whether Mobay's new insecticide NTN 33893 (seed treatment) reduces transmission of BYDV by Rhopalosiphum padi.

Infective (PAV) R. padi were disseminated in 1 m x 1 m field cages of Arapahoe winter wheat seedlings (3-leaf stage), the seed of which had been treated with NTN. Plants in the cages will be tested for BYDV infection and grain yields from those plots will be compared with non-infected check plots.

Fuller, B. W., T. L. Anderson, R. W. Kieckhefer, T. Wang, W. W. Chambers. 1991. Russian wheat aphid control: seed and rescue treatments. Insect. and Acara. Tests. Vol. 16.

C. Studies are being initiated to evaluate the potential for biological control of viruliferous aphids using coccinellids. Population dynamics of the aphid vector-BYDV systems will be studied in small grains.

1991 BARLEY YELLOW DWARF VIRUS WORKSHOP

Name: Roger H. Ratcliffe
Institution/Location: USDA, ARS, Insect and Weed Control Research Unit, West Lafayette, IN

Research Objectives and Approaches:

Barley Yellow Dwarf Virus (BYDV) research is not included among the objectives of the current CRIS (CWU Number 3602-20240-008-00D established 3/18/91) because of the need to concentrate available resources on Hessian fly resistance. This report summarizes BYDV research conducted since 1988 under CWU Number 3602-20240-004-00 D that terminated on 9/30/91. Objectives of the research were to, 1) identify new genetic sources of resistance to BYDV for development/deployment in wheat breeding programs for the eastern soft winter wheat region, 2) investigate the mechanism(s) of resistance, and 3) identify the biochemical/molecular basis for resistance in cereals to BYDV. Approaches included the use of established screening methods to identify Agropyron germplasm with BYDV resistance, and the use of an electronic system to monitor aphid feeding behavior and an enzyme-linked immunosorbent assay (ELISA) to quantify virus titer in plants, to determine if resistance was due to failure of aphids to innoculate plants, or to failure of the virus to establish itself within the plant.

Status of Research since 1988:

Research with the bird cherry oat aphid, (BCOA) Rhopalosiphum padi (L.) and English grain aphid, (EGA) Sitobion avenae (F.) vectors of BYDV, showed that infected aphids caused greater yield loss in wheat than non-viruliferous aphids, but non-viruliferous aphids, particularly late infestations of EGA, also caused yield loss. Seed number increased with increasing aphid infestation, but net yield was lower because grains were shriveled. Electronic monitoring of aphid feeding showed that EGA fed better, had faster and longer phloem contact, shorter development time, greater fecundity, and greater intrinsic rate of natural increase on infected than on healthy plants. Two symptom types ('notch') and ('red') were selected from the MAV isolate of BYDV. EGA developed better, and acquired virus with greatest efficiency from plants with the 'red' subculture . Studies on competition between aphid species showed that interspecific competition between EGA and BCOA did not affect the feeding behavior of BCOA, but resulted in fewer probes, shorter non-probing time, and longer probing/ingestion time for EGA. Conspecific competition caused an increase in non-probing time and a decrease in probing/ingestion time for BCOA, but not EGA. The longevity and fecundity of both species decreased with increasing aphid numbers. Wheat plants infested with Hessian fly (HF), Mayetiola destructor (Say), were generally more attractive to BCOA than uninfested plants, however, HF infestation did not significantly affect aphid reproductive and post-reproductive periods, longevity, and progeny.

Research Summary: 1991 Barley yellow dwarf virus workshop - USDA-ARS

Names: Jeffrey R. Vincent, Richard M. Lister, Mark J. Young and Brian A. Larkins

Institution and location: Departments of Botany and Plant Pathology, and Biology, Purdue University, W. Lafayette, Indiana, 47907

Research objectives and approaches: The primary objective of these studies is to understand the genomic basis for barley yellow dwarf virus (BYDV) biological functions. Toward this goal, cDNA libraries from three BYDV serotypes have been constructed. These libraries represent both Group 1 and Group 2 BYDV serotypes: specifically an MAV and a PAV serotype from Group 1, and an RPV serotype from Group 2. cDNA clones representing the genome of each of these BYDV serotypes have been identified, and sequenced. These libraries thus are the foundation of the genomic work and also have provided cDNA probes for diagnostic, epidemiological and biological cross-protection studies. From the sequencing of clones representing each of the BYDV serotypes, the genomic organization of the MAV-PS1 and P-PAV isolates were determined to be identical, while that of the NY-RPV isolate was determined to be like that of other luteoviruses such as potato leafroll virus and beet western yellows virus. The genomic work on these viruses is continuing toward the development of full-length cDNA clones which are capable of producing infectious synthetic BYDV RNA.

In the course of characterizing the BYDV genomes, coat protein genes for all three serotypes were identified, and cloned. These clones have since been used to construct plant transformation vectors with the potential of establishing coat protein-mediated resistance to BYDV in cereals. Future studies on BYDV genome structure and function will continue to evaluate mechanisms for the biotechnological control of BYDV.

Relevant publications:

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Young, M. J., L. Kelly, P. J. Larkin, P. M. Waterhouse and W. L. Gerlach. 1990. Infectious *in vitro* transcripts from a cloned cDNA of barley yellow dwarf virus. *Virology* 180,372-379.

Fattouh, F. A., P. P. Ueng, R. M. Lister and B. A. Larkins. 1990. Luteovirus relationships assessed by cDNA clones from barley yellow dwarf viruses. *Phytopathology* 79,1353-1358.

Research Summary: 1991 USDA/ARS workshop on barley yellow dwarf virus

Names: Fujiang Wen, Jianying Peng, Richard M. Lister, Jeffrey R. Vincent, Chih-Hsien Lei, Brian A. Larkins, and Thomas K. Hodges

Institution and location: Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907

Research objectives and approaches: We are attempting to transform cereal plants with BYDV coat protein genes, so that the resultant transgenic plants exhibit coat-protein mediated resistance to infection. Because coat protein-mediated resistance parallels cross protection in many aspects we have also examined classical cross protection effects between selected BYDV isolates. Initially, expression of the NY-RPV 22 kD coat protein gene was validated using a construct containing it to transform potato via the Agrobacterium Ti plasmid system. Recently, however we have regenerated plants from protoplast cultures of two BYDV-susceptible japonica rice varieties, Radon and Baldo, and established an efficient gene transformation system for them *via* direct DNA uptake. Protoplasts were transformed with BYDV coat protein cDNA or the *gus*A gene by PEG-mediated DNA uptake. Antibiotic-resistant calli were regenerated into plants.

Current Situation: Three plasmid constructs pPUR, pAct-GUS, and pAeiGUS, all containing the *gus*A gene but driven by the CaMV 35S, rice actin, or maize *Adh*-1 promoters, respectively, were each used, together with plasmid pKAN (containing the *neo* gene), for co-transformations of protoplasts of Baldo rice by PEG-mediated DNA uptake. After selection on medium containing the antibiotic G-418, 21% of the surviving calli exhibited GUS activity. Fluorometric assays revealed that all constructs expressed *gus*A in the transformed calli, with pAct-GUS expression being highest and pPUR lowest.

Protoplasts of both Radon and Baldo varieties have been used in co-transformation experiments using pKAN and the cDNA encoding the coat protein of the NY-RPV isolate of BYDV. One hundred and eighty Radon plants have been regenerated from G-418-resistant calli. We have screened 147 plants by Southern analysis, and found that seven were transformed with the coat protein cDNA. For one of these seven, tests by Northern analysis showed that the introduced coat protein cDNA was also transcribed into RNA. Further work is in progress to study the expression of the introduced coat protein gene, and the resistance of the transgenic plants and their progenies to BYDV infection.

Future Plans: Future work will investigate enhancing expression and directing it to phloem, using constructs derived from various BYDV isolates. Other BYDV genes will also be tried, and the studies extended to other small grain cereals.

Publications:

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Research Summary: BYDV workshop- USDA/ARS

Name: Mark Young

Institution and location: Department of Biological Sciences, Purdue University, West Lafayette, In 47907

BYDV research background:

Previous to joining the faculty at Purdue in May 1991, I worked on BYDV at CSIRO Division of Plant Industry, Canberra Australia. In order to overcome some of the inherent difficulties of working with BYDV I (1) developed a monocot protoplast assay system for the expression of the virus and (2) constructed a full-length BYDV cDNA clone from which infectious *in vitro* transcripts are produced. The cell culture system allows us to establish viral infections using intact virion particles or isolated RNA as inoculum in a wide range of monocots. As little as 5ng of input BYDV is required to establish an infection. High levels of infection are observed 48 hours post inoculation and BYDV replication can be followed using immunofluorescence, Northerns, and ELISA assays. Progeny virus particles arising in protoplasts can be transferred to whole plants by feeding BYDV aphid vectors on infected protoplast extracts. A full length cDNA clone of the PAV-BYDV serotype was constructed using cDNA and PCR cloning technology. T7 RNA polymerase directed transcription of the full length cDNA clone results in transcripts which are infectious when introduced into our protoplast system.

While at CSIRO I used the protoplast system in combination with the infectious BYDV cDNA to:

(1) Examine BYDV gene expression and regulation.

(2) Design and test synthetic resistance genes to interfere with BYDV replication.

(3) Develop a novel system for foreign gene expression in monocots using BYDV regulatory elements.

Current Research Objectives:

My long term research objective is to understand how viral gene expression causes disease. I am currently focusing on defining the viral determinants that control BYDV vector specificity and host range. To accomplish these objectives, the following approaches are being pursued:

(1) The continued characterization of BYDV gene expression and regulation.

(2) Over expression of viral gene products using the baculovirus expression for biochemical and structural studies.

(3) The construction of infectious cDNA clones of related viruses which share similar genomic organizations as BYDV, but differ significantly in their biological properties. By exchanging genomic segments between these related, but biologically distinct viruses, we hope to define the viral determinants for host range and vector transmissibility.

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